



MSU 4.1-587  
Appl. No. 10/074,499  
November 10, 2006

**IN THE UNITED STATES PATENT AND TRADEMARK OFFICE**

Appl. No. : 10/074,499 Confirmation No. 4246

Applicants : Evangelyn C. Alocilja and  
Zarini Muhammad-Tahir

Filed : February 13, 2002

Title : CONDUCTIMETRIC BIOSENSOR DEVICE,  
METHOD AND SYSTEM

TC/A.U. : 1641

Examiner : Leon Y. Lum

Docket No. : MSU 4.1-587

Customer No. : 21036

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Commissioner for Patents  
P.O. Box 1450  
Alexandria, VA 22313-1450

**DECLARATION UNDER 37 C.F.R. § 1.132**

Dear Sir:

Evangelyn C. Alocilja states as follows:

(1.) That she is an inventor of the invention in the  
above entitled application.

(2.) That she is an Associate Professor of Biosystems Engineering at Michigan State University, East Lansing, Michigan 48824.

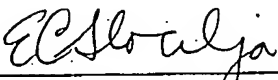
(3.) That the conductive polyaniline polymers synthesized by oxidative polymerization of aniline monomers, as described in Example 1 of the above entitled application, form polymer strands.

(4.) That these conductive polyaniline polymers strands of Example 1 are reacted directly with the antibodies, without using glutaraldehyde.

(5.) That these conductive polyaniline polymers synthesized by oxidative polymerization of aniline monomers are formed in the absence of any conductive particles.

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(6.) That the undersigned declares further that all statements made herein of her own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issuing thereon.

  
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Evangelyn C. Alscilja

Date: 11/10/06

## Polyaniline label-based conductometric sensor for IgG detection

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Accepted 13 May 1996

### Abstract

A new method based on a biochemical binding system is developed for detecting the presence and measuring the concentration of a definite analyte in fluids. The conductometric scheme of detection has been employed for detection of the binding reaction of immunoglobulins. Two identical pairs of gold interdigitated electrodes deposited on the insulating solid support have been used as a detector. An electrically conducting polymer, namely polyaniline, has been proposed as a label for immunosensor development. A set of water-soluble forms of polyaniline with different molecular weights and oxidation levels have been prepared by chemical oxidation of aniline and characterized using gel-permeation chromatography. The acceptability of different kinds of polyaniline, and the selectivity and sensitivity of sensor response have been studied. The method of antibody detection in competitive electroimmunoassay has been developed. The lowest antigen concentration which may be detected in the competitive mode was found to be about 500 ng/ml. The results of impedance spectroscopy measurements obtained for different states of the electrodes are analyzed in order to clarify the mechanism of the sensor response.

**Keywords:** Electrochemical immunoassay; Immunosensor; Polyaniline; Electroactive label

### 1. Introduction

Due to their specificity and high sensitivity, immunoassays are commonly used for analysis of clinically important compounds contained in biological liquids, analysis of environmental pollutants and in the food industry.

Radioisotopic labels have played an important role in the development of immunoassays since Yallow and Berson introduced radioisotopes in immunoassays in 1959 [1]. However, disadvantages, associated with the use of radioactive materials have led to development of techniques employing non-isotopic labels. Enzyme-linked immunosorbent assays (ELISA) [2], fluorescent [2,3] and chemiluminescent [4,5] immunoassays are being commonly used for clinical analysis as well as for fundamental studies.

Despite the excellent sensitivity of such methods (picograms to nanograms of analyte per milliliter), their use is limited because of the necessity for complicated and expensive equipment, laboriousness and relatively

long time of analysis. Alternative techniques are therefore being developed for express immunological testing. One of the most attractive approaches in this field is to detect immunological reactions electrochemically. An electrochemical immunoassay can be carried out when one of the reactants is labelled with an electroactive substance [6–8]. Differential pulse voltammetry and adsorptive stripping voltammetry are two extremely sensitive electrochemical methods commonly used for determination of electroactive organic compounds and metal ions. Various metals such as nickel [9] and cobalt [10–12] have been used in voltammetric immunoassays for determination of antibody concentration by monitoring the change in the reduction currents of the metal–protein complex. Cais et al. [12] employed mercuric and platinum labelled haptens for metalloimmunoassay. The sensitivity of the methods proposed varies from metal to metal and under optimized conditions gives the possibility of determining 0.03–2 µg of metallohapten/ml.

Nitro groups and mercuric acetate have been also employed as labels for estradiol. The method is based on introducing an electroactive group detectable by differential pulse polarography into an antigen electrochemically in-

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active in the potential range employed [13,14]. The detection limit of these methods for labelled estriol is 60 ng/ml.

Indium has been involved in a heterogeneous voltammetric immunoassay as a label for serum albumin [15] and differential anodic stripping voltammetry has been used as a function method. The detection limit for an electrochemical immunoassay in this format was 5 µg/ml. Hayes et al. [16] have demonstrated the concept of simultaneous dual analyte immunoassay based on two different metal ion labels: bismuth and indium attached to HSA and IgG through a bifunctional chelating agent. Limits of detection for HSA and IgG are 1.8 and 0.6 µg/ml respectively.

Sugawara et al. [17] proposed using daunomycin as an electroactive labelling reagent for biotin. The detection limit for daunomycin-labelled biotin which can be detected voltammetrically is  $3 \times 10^{-9}$  mol/l.

Metallocompounds [18,19] have been used as labels due to their ability to act as mediators of an enzyme, and therefore, catalytic current can be inhibited due to immunological reaction. This can be reversed by the addition of free antigen that competes for the available antibody binding sites. The detection limit of the method proposed is about 5 µM of hapten.

In the present study the use of conducting polymer-polyaniline (PANI) as label for immunoelectrochemical immunoassay was tested. Polyaniline attracted our attention because of its unique electrical and chemical properties: it is generally recognized as the only air-stable conducting polymer, has very high electrical conductivity in the protonated state [20] and can be bound easily to most proteins. We suggest that the binding reaction between a PANI-labelled immunospecific agent in the solution and another one immobilized on the interdigitated planar electrodes should give a substantial conductometric response.

In our previous work the possibility for such an experimental approach has been shown [21,22].

## 2. Experimental

### 2.1. Reagents

Aniline, glutaraldehyde and goat anti-rabbit IgG were purchased from Sigma. Rabbit IgG was obtained from DIAM Ltd. (Moscow, Russia). Sephadex G-100 and Sephacryl S-300 were purchased from Pharmacia. *N,N*-Dimethylformamide was obtained from Fluka. All reagents used were of analytical reagent grade.

### 2.2. Synthesis of polyaniline

The water-soluble polyaniline was synthesized according to the following procedure. Oxidative polymerization of aniline was performed by adding different amounts of

ammonium peroxodisulfate (0.2 mM; 0.4 mM; 0.6 mM; 0.8 mM) into the 0.4 M aniline solutions in 1 M HCl while stirring at room temperature for 30 min. After the polymerization, the product was separated by filtration and washed with 5% aqueous ammonia. The polyaniline base obtained was dissolved in 5 ml *N,N*-dimethylformamide (DMFA) and filtered. Then the filtrate was mixed with 2 ml 2 M HCl in DMFA and filtered again. After the solvent had been evaporated, the sediment was dissolved in water.

### 2.3. Conjugation procedure

Polyaniline fractions obtained as a result of gel-filtration on TSK gel Toyopearl HW-40 were treated with 0.1% glutaraldehyde under stirring for 1.5 h and then 0.5 ml aliquots of rabbit IgG (1 mg/ml) were added to each polyaniline fraction coupled with glutaraldehyde. Non-specific antibodies were employed for the reference experiments. The duration of the incubation procedure was 1.5 h at room temperature. To inactivate non-reacted aldehyde groups, 0.3 ml of 0.1 M Tris-glycine buffer (pH 9) was added to the reaction mixture and incubated while stirring for 0.5 h at room temperature. Finally, the conjugated material (PANI-IgG) was separated from unconjugated components by gel-filtration on G-100 Sephadex.

Gel-permeation chromatography of PANI and PANI-antibody conjugate was monitored by an LKB 2138 Uvicord S flow monitor (LKB, Sweden).

### 2.4. Electrodes and measuring system

Au/Cr electrodes were evaporated onto Si/SiO<sub>2</sub>/Si<sub>3</sub>N<sub>4</sub> slides using a conventional thin film processes. Two identical pairs of comb-like electrodes were patterned by means of photolithography so that 20-µm gaps between the adjacent electrodes were formed. The experimental set-up for conductometric measurements consists of a glass cell, magnetic stirrer, holder of the electrodes and electronic units. To measure the impedance response of the working pair of electrodes in comparison to a reference one, a differential preamplifier was switched as shown in Fig. 1. In this case the frequency was fixed at 20 kHz and the output voltage was read as a function of antibody concentration in the immersing electrolyte. Impedance spectroscopy was carried out using a standard resistance box in series with the tested electrodes and frequency generator. By adjusting the box to make the AC-voltage drop by half of the generator output, the magnitude of the electrodes impedance can be read from the box. A selective AC-nanovoltmetre (Unipan-237) was used for monitoring the AC voltage drops across the electrodes. The impedance spectra were obtained by changing the frequency of the generator and respective tuning of the selective nanovoltmetre. The generator output was always fixed at 60 mV. This prevents redox proc-

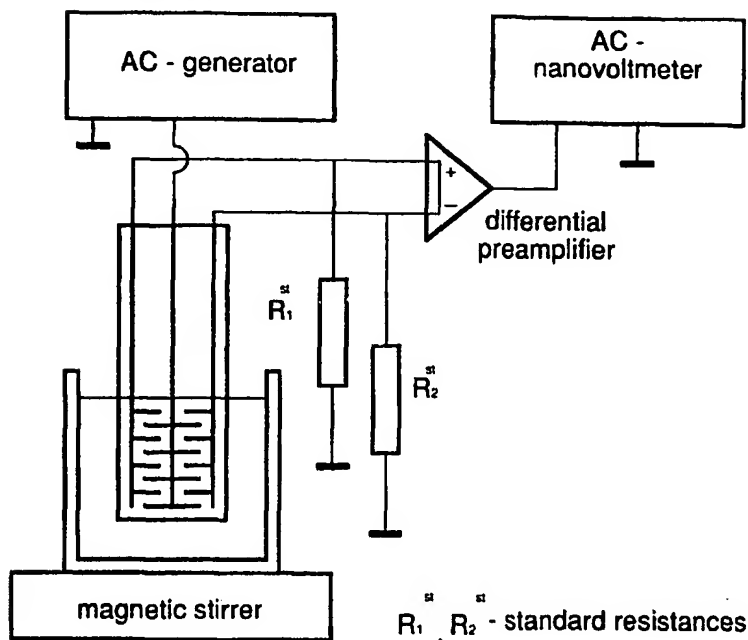


Fig. 1. Schematic diagram of the measuring system for the registration of the differential AC responses.

esses at the electrodes on the one hand and gives high signal/noise ratio, on the other hand.

### 2.5. Immobilization procedure

Anti-rabbit goat IgG was immobilized onto the surface of the working electrodes by passive adsorption as an antigen (AG). The adsorption was performed by immersing the electrodes in 20 mM phosphate buffer solution containing 140 mM NaCl (PBS) and 1 mg/ml anti-rabbit goat IgG for 2 h at room temperature (or 24 h at 4°C). The electrodes were then incubated in 2 mg/ml BSA solution in order to reduce non-specific adsorption of the labelled components during the subsequent immunoassay.

### 3. Results and discussion

The present study employs polyaniline molecules as a label for one of the binding pair components. Prepared according to the described procedure, samples of water-soluble PANI with different oxidation levels have been studied by gel-filtration on TSK gel Toyopearl HW-40 and Sephacryl S-300 to characterize low and high molecular weight fractions, respectively. Each of the synthesized polyanilines contained fractions with molecular weight 10 kDa to 1 MDa. A variety of the polyaniline forms obtained by gel-filtration was used for labelling IgG.

For electrical immunoassays prepared conjugates were added into the solution immersing the electrodes with immobilized complementary antibodies. The impedance of the electrodes was found to be highly sensitive to the

presence of labelled antibodies. The responses reached saturation in about 5 min, so in all the experiments transducers were allowed to stay for 5 min after the immunoreagent had been added, prior to read-out of the output signal. The steady state responses were in proportion with conjugate concentration.

Conjugate of non-specific antibodies with polyaniline were employed for control experiments. The sensor response on the addition of the specific conjugate was three times larger than the non-specific one. The minimum quantity of labelled antibodies which can be easily detected is 50 ng/ml (Fig. 2).

Fig. 3 depicts efficiency of PANI-labels having different molecular weights and oxidation levels. The data shown correspond to the frequency 20 kHz although similar dependences were observed in the wide frequency range (0.3–80 kHz). As can be seen, the response increases in proportion to the molecular weight of the label except the highest value (1 MDa). This fact seems to be quite reasonable because the total amount of the conductive polymer near the electrode surface should be in direct proportion to its molecular weight if the amount of the conjugate specifically bound onto the electrode surface is constant. Nevertheless, when the molecular weight of the label is much higher than that of the antibody, conformation of the latter is likely to be changed and the bio-specific binding is suppressed. The maximum specific response was observed for the PANI fraction of molecular weight 45 kDa.

The influence of the oxidation level of polyaniline on the sensor response was also determined. It was found that the lower the oxidation level, the larger the sensor

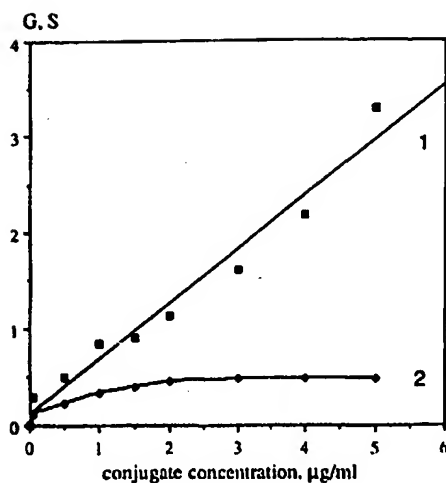


Fig. 2. Dependence of the sensor response on specific (1) PANI-IgG and non-specific (2) conjugate concentration. The steady-state signal was read out 5 min after the conjugate addition. 45 kDa PANI fraction synthesized in the presence of 0.2 mM ammonium peroxodisulfate is used for IgG labelling. Goat anti-rabbit IgG immobilized on the surface of electrodes.

response. Since the maximum response was found for PANI with a molecular weight of 45 kDa synthesized in the presence of 0.2 mM ammonium peroxodisulfate, this fraction was selected for the further experiments.

The experiments performed in the competitive mode demonstrate the detection of the unlabelled IgG with the proposed sensor. Constant amounts of PANI-IgG conjugate mixed with different amounts of the unlabelled IgG

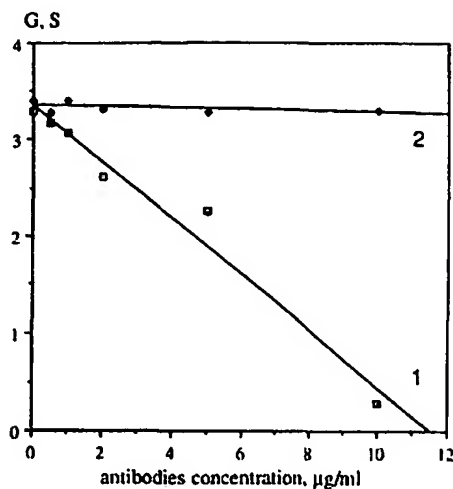


Fig. 4. Reduction of the immunosensor response on addition of 5 μg/ml PANI-IgG versus the concentration of the unlabelled (1) rabbit IgG, (2) non-specific antibodies added simultaneously. 45 kDa PANI fraction synthesized in the presence of 0.2 mM ammonium peroxodisulfate is used for IgG labelling. The steady-state signal was read out 5 min after the conjugate addition. Goat anti-rabbit IgG immobilized on the surface of electrodes.

have been added into the electrochemical cell. A decrease of the sensor response due to the presence of unlabelled component has been observed; the decrease was proportional to the amount of analyte. It has been shown that 500 ng/ml IgG can be easily detected in a competitive electroimmunoassay (Fig. 4).

To understand the mechanism of the sensor response we carried out impedance spectroscopy subsequently for: (i) dry electrodes, (ii) bare electrodes immersed in various electrolytes and (iii) electrodes with immobilized AG in PBS before and after PANI-AB addition.

The impedance was found to decrease substantially when the electrodes were dipped into an electrolyte. The adsorption of AG onto the electrodes did not alter the impedance of the electrodes substantially. This may be attributed to loose packing of the protein molecules in the adsorbed layer. When the complementary AB labelled with PANI was added into the immersing solution, a reproducible change of the impedance spectrum was observed. Binding of the PANI labelled antibodies to the electrode surface leads to the almost proportional decrease of the impedance in a wide frequency range. This rather unusual result may be explained in terms of the fractal dimension of the electrode coated with a polymer layer. Electrically active PANI molecules bound to the electrode modify the interface making it rougher on the nanometer scale. This roughness may account for the electrical characteristics as the fractal dimension of the interface is higher than 2 [23]. More simply, the responses observed on binding conducting polymer to the electrode surface may be explained by an increase of ef-

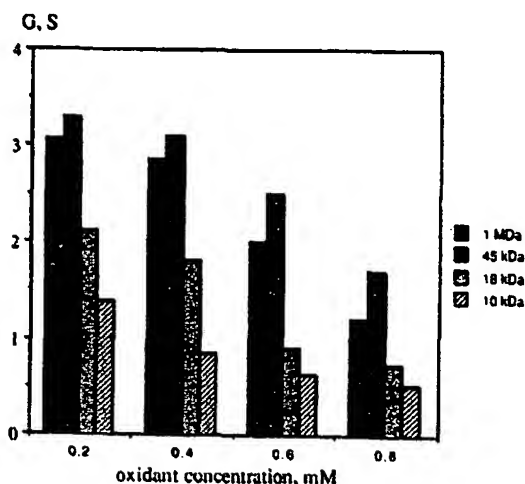


Fig. 3. Immunosensors responses on 5 μg/ml PANI-IgG addition for different molecular weight and oxidation levels of the label used. The steady-state signal was read out 5 min after the conjugate addition. Goat anti-rabbit IgG adsorbed on the surface of electrodes.

fective surface and, consequently, the interfacial capacitance of the electrode when it is surface coated with the conductive polymer.

In contrast, an active charge transport between adjacent electrodes, directly through the polyaniline chains, is unlikely to be the case or, at least, its contribution to the total impedance is negligible. If the PANI chains were forming conductive pathways between adjacent electrodes, the relative decrease of the impedance would be higher at the lowest frequencies, where an active electron current predominates over a capacitive one. Therefore, the conductivity of the polyaniline, which is known to be essentially pH-dependent, does not contribute to the impedance of the transducer directly. This is because pH has no crucial effect on the efficiency of the proposed method.

#### 4. Conclusions

The newly prepared water-soluble forms of polyaniline offer good possibilities for immunosensor design. The present study employs polyaniline molecules as labels for one of the components of the biospecific binding pair. When another component is immobilized onto thin film interdigitated electrodes, the biospecific interaction can be easily monitored by AC conductometric technique.

For such an immunoassay the most suitable form of polyaniline was found to be the fraction with molecular weight of about 45 kDa, synthesized in the presence of 0.2 mM ammonium peroxodisulfate.

At least 500 ng/ml of unlabelled IgG can be detected in the competitive electroimmunoassay with the proposed method. Therefore, the method proposed is 3 orders less sensitive than ELISA, although it is comparable with the sensitivity of existing electrochemical immunoassays. The time of analysis according to the proposed method is a few minutes only.

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#### Biographies

**Tatiana A. Sergeyeva** graduated from Kiev State University in 1993 and received an M.Sc. in immunology. She is now doing her post-graduate course in the Institute of Molecular Biology and Genetics; her present scientific interests include development of affinity-based and enzyme sensors.

**Nikolay V. Lavrik** graduated from Kiev Polytechnical Institute in 1987 and received an M.Sc. in microelectronics. Since that time he has worked at the Institute of Semiconductors Physics in the field of Molecular electronics and Microelectronics. Currently his main field of interest is the use of ordered organic films in biosensors and chemically sensitive devices. He obtained his Ph.D. degree in 1994 (semiconductors physics).



**Sergey A. Piletsky** graduated from Kiev State University in 1986 and received an M.Sc. in bioorganic chemistry. He obtained his Ph.D. degree in 1991 (bioorganic chemistry). Since that time he has worked in the Institute of Molecular Biology and Genetics. His main field of interest is development of template sensors and biosensors.

**Alexander E. Rachkov** graduated from Kiev State University in 1978. Since that time he has worked in the Institute of Molecular Biology and Genetics. He obtained his Ph.D. degree in 1991 (molecular biology). His present

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**Anna V. El'skaya** graduated from Donetsk Medical Institute in 1963. She had her post graduation course in Institute of Biochemistry, Kiev. She obtained her Ph.D. degree in 1968 and Doctor degree in 1975. Since 1983 she is Full professor, Kiev State University. Since 1992 she is Academician of Academy of Sciences of Ukraine. Her main field of interests are translation mechanisms of genetic information and biosensors development.

PAPERS

## Synthesis and Examination of Polyanilines as Labels in Immunosensor Analysis

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Received May 12, 1994

**Abstract**—A number of polyaniline preparations differing in oxidation level and molecular sizes were obtained and characterized. A method of polyaniline conjugation with immunoglobulin was developed, and the possibility of using polyaniline as a label in the development of an immunosensor with conductometric measuring circuit was demonstrated.

Current needs of medicine, environmental monitoring, and biotechnology pose a problem to the development and creation of analytical systems that combine high specificity and sensitivity of determinations with convenience, ease, and compactness. These systems successfully solve the problems of the diagnostics and treatment of diseases and the problems of environmental monitoring and process control; they allow rapid data acquisition and processing.

Immunochemical methods, primarily radioimmunoassay and enzyme-linked immunosorbent assay, are widely used in biotechnology, medicine, and environmental sciences along with traditional physicochemical and biochemical methods. However, all of these methods are characterized by a long analysis time, the impossibility of performing continuous analysis, a single use of solid phases, complexity, and laboriousness.

Immunosensors are novel biotechnological devices, which are developed to overcome these disadvantages. In immunosensors, a transducer of a physical signal, which appears in the process of biochemical recognition of a certain agent, is in direct contact with a highly specific immobilized receptor (antigen or antibody depending on the purpose and scheme of the analysis).

The approach under development is based on the use of a sensor that combines the sensitivity of an electrochemical detector with the specificity of an antigen-antibody reaction.

The aim of this work was to synthesize electrochemically active compounds suitable for labeling antibodies and to apply these compounds to the development of a conductometric immunosensor.

### EXPERIMENTAL

To synthesize polyanilines with different oxidation levels, 23, 46, 92, or 116 mg (0.2, 0.4, 0.6, or 0.8 mM, respectively) of ammonium persulfate in 4 mL of 1 M HCl was added to a mixture of 0.4 mL of aniline and 6 mL of 1 M HCl, and the contents were incubated for 30 min at room temperature. The precipitate obtained

was washed several times with a 5% ammonia solution, dissolved in 5 mL of *N,N*-dimethylformamide (DMF), and filtered; 2 mL of 2 M HCl in DMF was added to the filtrate, and the contents were filtered again. The solvent was removed with a rotary evaporator at 70°C. The polyaniline precipitate was dissolved in 1 mL of water.

Polyanilines were fractionated according to molecular mass by gel filtration on TSK-gel Toyopearl HW-40 and Sephacryl S-300 columns (to characterize fractions of low and high molecular masses, respectively) in a 20 mM phosphate buffer (pH 7.5) containing 140 mM of NaCl and 10 vol % of DMF.

**Conjugation of antibodies with polyaniline.** Five microliters of 25% glutaric dialdehyde was added to 400  $\mu$ L of a polyaniline solution, and the mixture was incubated with continuous stirring at room temperature for 1.5 h. A solution (400  $\mu$ L) of specific (anti-IgG) or nonspecific (IgG) antibodies (for blank experiments) with a concentration of 1 mg/mL was added to the mixture, and the contents were further incubated for 1.5 h under the same conditions.

**Antibody immobilization.** The immobilization of IgG at the detector surface was performed by physical sorption from a solution with a concentration of 5 mg/mL at 4°C overnight or at room temperature for 2 h.

**Measuring circuit.** A measuring setup involved a GZ-112/1 audio signal generator and a load resistor (100  $\Omega$ ) as described elsewhere [1]. Plane electrodes involving an insulating substrate with a gold layer applied by vacuum deposition in so-called fingered geometry with the finger spacing of 20  $\mu$ m (Fig. 1) were used as a detector. An interaction of various substances with the detector surface caused changes in the electrical conductivity. An output signal taken at the electrodes came to a phase-sensitive nanovoltmeter of the Unipan 237 type.

### RESULTS AND DISCUSSION

The discovery of high electrical conductivity of polyacetylene [2] caused an increased interest in the search

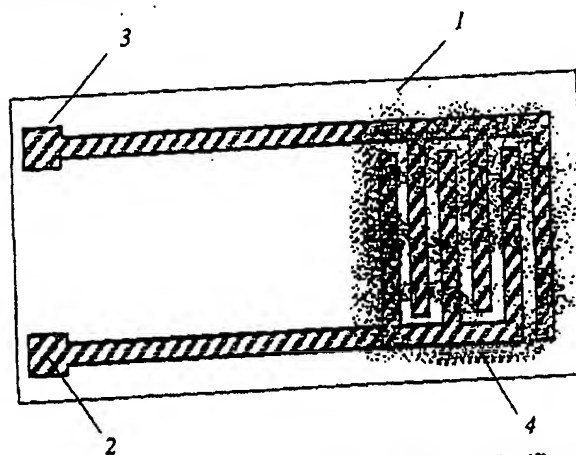


Fig. 1. Schematic diagram of a plane electrode: (1) insulating solid substrate; (2) anode; (3) cathode; and (4) biologically active material immobilized.

for polymeric compounds that have these properties and in an intensive study of these compounds [3-5]. Polyaniline, which has some advantages over other electrochemical labels, occupies a special place among conducting polymers. Polyaniline is one of a few conducting compounds stable in an oxygen atmosphere, contains functional groups suitable for the conjugation with proteins, and can be easily synthesized from available materials.

Polyanilines were obtained by the polymerization of aniline in the presence of ammonium persulfate according to the procedure mentioned above. A completely reduced polyaniline is known as leucoemeraldin; the most oxidized form is called pernigraniline, and an intermediate form, which is the most stable in air, is named emeraldin [3]. The product synthesized was green in an acidic medium and turned dark blue after treating it with an ammonia solution. These changes correspond to the transfer of an emeraldin salt into the base.

A number of water-soluble polyaniline forms, that differ in the oxidation level and molecular mass, were obtained by varying the ammonium persulfate concentration. Using gel filtration on TSK-Toyopearl HW-40 and Sephacryl S-300 columns to characterize fractions of low and high molecular masses, respectively, a high-molecular-mass (about 1 MDa) fraction and low-molecular-mass (10-45 kDa) fractions were separated from each of the synthesized polyaniline preparations. The fractions obtained were concentrated with a rotary evaporator and used later for the conjugation with antibodies to rabbit IgG.

Electrochemical properties of synthesized polyanilines were examined with the use of a conductometric measuring circuit. It was demonstrated that the polyaniline immobilization caused a significant (by one order of magnitude) increase in the electrical conductivity of the detector. The highest electrical conductivity was observed for the polyaniline polymerized in the presence of 0.2 mM ammonium persulfate. When analyzing the effect of ac frequency on the electrical con-

ductivity of polyaniline films, we found that the electrical conductivity was maximum at a frequency of 80 kHz. This fact indicates that a sensor response is formed by an ionic mechanism of conductivity rather than by an electron mechanism. This is also supported by the fact that an increase in ionic strength of the solution caused a decrease in sensitivity of the sensor. A 5 mM concentration of phosphate buffer solution was optimum for the measurements.

Conditions of the sensor response formation were optimized using a model system in which rabbit IgG served as the antigen, and goat antibodies to rabbit IgG were the antibodies. The detector with the antigens (rab-

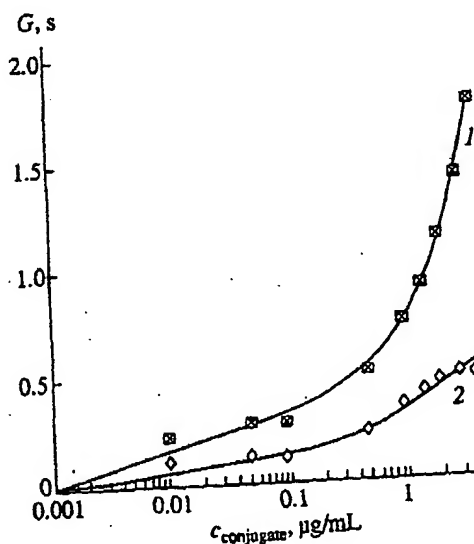


Fig. 2. Dependence of the sensor response on the concentration of added conjugates of polyaniline (45 kDa) with (1) specific (anti-IgG) or (2) nonspecific (IgG) antibodies.

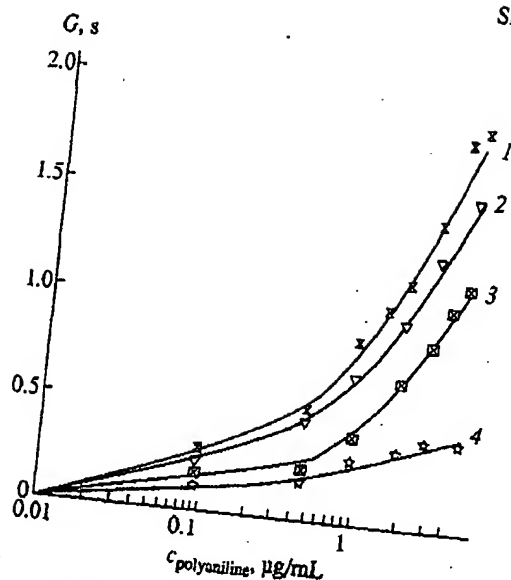


Fig. 3. Dependence of the sensor response on the oxidation level of a polyaniline label (molecular mass of the label was 45 kDa): (1-4) conjugates of specific antibodies with polyaniline synthesized in the presence of 0.2, 0.4, 0.6, or 0.8 mM ammonium persulfate, respectively.

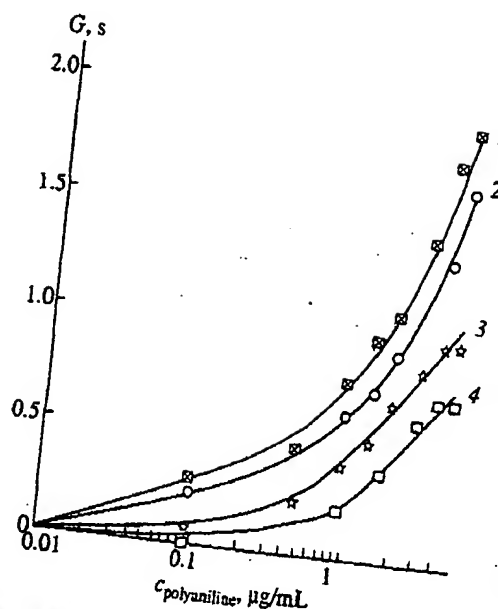


Fig. 4. Dependence of the sensor response on the molecular sizes of polyaniline: (1-4) conjugates of specific antibodies with polyaniline preparations with the molecular mass of 45 kDa, 1 MDa, 18 kDa, or 10 kDa, respectively.

bit IgG) immobilized at its surface by physical sorption is a biosensor. The method suggested operates on the following principle: a biospecific recognition occurs on the interaction of sorbed antigens with polyaniline-labeled antibodies specific to this antigen, and, as a consequence, an increase in the electrical conductivity of the system is expected because of the formation of a conducting layer at the biosensor surface. The increase in the electrical conductivity will be proportional to the concentration of labeled antibodies in a sample.

The study of an antigen-antibody reaction, one of the components of which was labeled with polyaniline, demonstrated that a specific signal was significantly higher than nonspecific signals on addition of a conjugate in the concentration range from 50 ng/mL to 5 µg/mL (Fig. 2). The dependence of the sensor response on the polyaniline oxidation level was studied (Fig. 3). For the polyaniline preparations with lower oxidation level (i.e., synthesized in the presence of 0.2 and 0.4 mM ammonium persulfate), the observed signal was significantly higher than level. The polyaniline preparation synthesized in the presence of 0.8 mM ammonium persulfate gave a signal that was almost no different from the background.

An increase in the signal with increasing molecular mass of polyaniline used as the label (Fig. 4) appears to be reasonable because polyaniline with higher molecular mass can form more contacts with neighboring polyaniline chains. A somewhat decreased signal for the conjugate of antibodies and polyaniline with a molecular mass of about 1 MDa can evidently be explained by

some steric hindrances for the interaction of an antigen with the antibody bonded to so bulky a label.

The method suggested for the detection of antigen-antibody interactions is rather simple and convenient. It is characterized by high sensitivity and short analysis time (the sensor response time is as short as 1 or 2 min); thus it appears to be of considerable promise for the development and creation of an immunosensor for determining antigens or antibodies in various biological fluids.

## CONCLUSION

A sensitivity level comparable to that of traditional enzymeimmunoassay procedures was attained with the use of a fraction of polyaniline (with a molecular mass of 45 kDa) synthesized in the presence of 0.2 mM ammonium persulfate. In this case, the response time of this immunosensor device was as short as a few minutes.

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